Process for producing penicillin

Field of the invention

The present invention relates to isolated nucleic acid molecules which encode a novel Penicillium chrysogenum protein, to vectors which contain such a nucleic acid molecule, to host cells which have been transformed with such a nucleic acid molecule or vector, and to a process for producing penicillin using such transformed cells.

Background to the invention

Penicillin is a natural metabolite which is obtained on an industrial scale by fermenting the filamentous fungus Penicillium chrysogenum (termed P. chrysogenum in that which follows). In addition to this, penicillin G and penicillin V form important precursors for a number of semisynthetic penicillin antibiotics. The penicillin substance class is of great therapeutic importance. Aside from improvements in process technology, achieving yield increases in industrial penicillin fermentation is essentially based on continuously improving strains genetically. The transformation of production strains with specific genes which display a potential for increasing production is becoming evermore prominent in modern methods for achieving this strain improvement. An understanding of biochemical interrelationships in penicillin biosynthesis suggests that a small group of known penicillin biosynthesis genes possess strain improvement potential. In experiments, amplifying, i.e. increasing the copy number, of these known genes does in fact sometimes result in a significant improvement in the productivity of a production organism. However, the group of known genes which can be predicted, on the basis of considerations of scientific plausibility, to have strain improvement potential is very small. However, it can be assumed that, in addition to these known biosynthetic genes, there is an unknown number of other genes which, by means of amplification, would likewise achieve a production-increasing potential. The function of these genes is frequently not known since the entirety of the cellular processes having an influence on penicillin biosynthesis is at present still not at all well understood. Strategies for identifying additional genes which have production-increasing potential are therefore of great importance.

The important penicillin biosynthesis genes, i.e. ACV synthetase (ACVS), isopenicillin-N (IPN) synthase and acyl CoA : IPN acyl transferase have been known for a relatively long time. The central enzyme is ACVS, a nonribosomal peptide synthetase (NRPS) which catalyses the formation of the tripeptide ACV. It is only in recent years that it has become known that, in some microorganisms, NRPS has to be "loaded" with phosphopantethein in order to be brought into an active form.

4'-Phosphopantethein transferases (PPTases) catalyse the transfer of the 4'-phosphopantethein (Ppant) group from coenzyme A (CoASH) to the hydroxyl function of the side chain of a conserved serine residue which is located in Ppant-dependent carrier proteins. In this connection, the carrier protein, universally abbreviated to XCP, is converted from the catalytically inactive apo form into the catalytically active holo form. The reaction is Mg2+-dependent and forms 3'-5'-ADP as a by-product. There are a variety of Ppant-dependent biosynthetic pathways. Fatty acid biosynthesis, in which the acyl carrier protein (ACP) binds the intermediates, is found in every cell. Many antibiotics and natural products, such as cyclosporin and the ß-lactams, are produced by nonribosomal peptide synthetases (NRPS) or polyketide synthases (PKS) which contain peptidyl-carrier proteins (PCP) or, respectively, ACP. Finally, a specialised peptide synthetase can be found in fungi and some plants in a biosynthetic route which leads to lysine. All these biosynthetic pathways share in common the feature that the carrier proteins involved are phosphopantetheinylated by PPTases and thereby converted into the active form. While the PPTases are essential factors for these processes, the genes which encode them are in many cases still unknown. Neither a PPTase of this nature nor the corresponding gene has thus far been described in P. chrysogenum.

Discovering such a previously unknown, PPTase-encoding gene in P. chrysogenum consequently forms a central object of the present invention. One object of the present invention is thus to provide a nucleic acid, and vectors, which encode a novel P. chrysogenum protein and which can be used for transforming a P. chrysogenum host cell such that this host cell is able to supply penicillin in good yields. Another object of the present invention is to provide such a transformed host cell. Finally, a further object of the present invention is to provide a process for preparing penicillin while using the said transformed host cell.

Figures

Fig. 1 shows the amino acid sequence (SEQ ID No. 1 = sequence identity No. 1) of a novel P. chrysogenum protein, which sequence is deduced from the nucleic acid molecule according to the invention (nucleic acid sequence in accordance with Fig. 2 or 4). The sequence is depicted proceeding from the N terminus to the C terminus.

Fig. 2 (SEQ ID No. 2) shows the genomic DNA sequence, including the 1 intron, of the coding region of the P. chrysogenum pptA gene from the translation start codon (ATG) through to the translation stop codon (TAA). The intron is underlined; the sequence is depicted as a single strand, proceeding in the 5' to 3' direction.

Fig. 3 (SEQ ID No. 3) shows the cDNA sequence of the coding region of the novel gene from the translation start codon (ATG) through to the translation stop codon (TAA); the sequence is depicted as a single strand proceeding in the 5' to 3' direction.

Fig. 4 (SEQ ID No. 4) shows the genomic DNA sequence of a SalI fragment of a genomic clone of the novel gene (the sequence is depicted as a single strand proceeding in the 5' to 3' direction). The translation start codon (ATG) and the translation stop codon (TAA) of the coding region are underlined and printed in bold; the intron is underlined.

Detailed description of the invention

A novel P. chrysogenum gene, which encodes a previously unknown protein in P. chrysogenum, is described within the context of the present invention. It is shown that the protein is a novel PPTase and that cotransformation experiments using this gene can result in strains which have penicillin titers which are high as measured by industrial criteria.

The novel gene can be isolated from the P. chrysogenum strain P2 = ATCC 48271 (obtainable under this number from ATCC, American Type Culture Collection, PO Box No. 1549, Manassas, VA 20108, USA). However, the novel gene can also be found in other P. chrysogenum strains. As an alternative, the nucleic acid and amino acid sequences or molecules which are present here can be prepared synthetically.

The gene encodes a protein which is 411 amino acids in length. The amino acid sequence is shown in Figure 1. In the gene, the coding region is interrupted by 1 intron, as can be seen in Figures 2 and 4.

The P. chrysogenum gene according to the invention is characterized as being a gene for a previously unknown PPTase, and designated the pptA gene, on the basis of functional tests (see Example 2).

One part of the subject-matter of the present invention is consequently an isolated nucleic acid molecule which encodes a protein which comprises the amino acid sequence as depicted in SEQ ID No. 1.

Such a nucleic acid molecule can consequently, for example, encode a protein which, in addition to the stated amino acid sequence (SEQ ID No. 1), also contains further amino acids, for example encode a fusion protein. Such fusion proteins can play a role, for example, when it is desired to prepare the novel protein in isolated form. The fusion moieties can, for example, increase stability or facilitate purification.

Within the context of the present invention, preference is given to a nucleic acid molecule according to the invention which only encodes an amino acid sequence as depicted in SEQ ID No. 1. Such a nucleic acid molecule can advantageously be employed for the purpose of producing penicillins, in particular penicillin V or G, as described below. Consequently, another part of the subject-matter of the present invention is a nucleic acid molecule according to the invention which encodes a protein which only possesses the amino acid sequence depicted in SEQ ID No. 1.

A nucleic acid molecule according to the invention is preferably a DNA molecule. Alternatively, the nucleic acid molecule can be an RNA molecule, in particular an mRNA molecule.

A DNA molecule according to the invention can, for example, be prepared by producing a genomic DNA library from the genome of the said P. chrysogenum strain ATCC48271. A genomic clone is identified by screening with homologous probes whose structures can be deduced from the described nucleic acid sequence of the gene as shown in Fig. 4.

Appropriate techniques are known from the literature (thus, for example, in T. Maniatis et al., Molecular Cloning - A Laboratory Manual, 1982, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA). The sought-after DNA molecule is located on a SalI fragment, of about 3.2 kb in size, of such a clone, which can be isolated or prepared using classical techniques. Such a fragment is depicted in Fig. 4. A preferred embodiment of the present invention consequently relates to a nucleic acid molecule according to the invention which comprises a base sequence as depicted in SEQ ID No. 4 or a base sequence which only differs from the sequence depicted in SEQ ID No. 4 because of the degeneracy of the genetic code. That is, according to the invention, the present invention also relates to those nucleic acid molecules which differ from the specifically listed sequences in that one or more of the listed codons is/are replaced with a, or several, different codon(s) such that the amino acid sequence of the encoded protein (SEQ ID No. 1) is not altered. This also includes the use of one (or more) alternative stop codons. This also applies to the other nucleic acid molecules which are described below. The nucleic acid molecule as depicted in SEQ ID No. 4 contains regulatory sequences (such as a promoter and a stop codon) and can advantageously be used, in particular in a vector, for transforming P. chrysogenum and thus for producing penicillin, in particular penicillin G or penicillin V.

The said SalI fragment of about 3.2 kb in size comprises, in particular, the coding moiety of the novel gene. This moiety is shown in Fig. 2 and contains 1 intron. Consequently, the present invention also relates to a nucleic acid molecule according to the invention which contains a base sequence as depicted in SEQ ID No. 2 or a base sequence which only differs from the sequence as depicted in SEQ ID No. 2 because of the degeneracy of the genetic code, as explained above. Such a nucleic acid molecule consequently corresponds to the genomic DNA sequence of the coding moiety of the novel gene. Other preferred embodiments of the present invention are those nucleic acid molecules which differ from that of SEQ ID No. 2 by the absence of the one intron.

Preference is consequently furthermore given to a nucleic acid molecule according to the invention which comprises a base sequence as depicted in SEQ ID No. 3 or a base sequence which differs from the sequence as depicted in SEQ ID No. 3 because of the degeneracy of the genetic code, as explained above. Such a nucleic acid molecule no longer contains an intron and, as such, can be equated with a corresponding cDNA sequence.

In addition, a nucleic acid molecule according to the invention (including a said cDNA molecule) can, for example, be prepared completely synthetically or partially synthetically. Standard techniques can be used to isolate RNA or mRNA molecules according to the invention from the microorganism P. chrysogenum or to prepare them synthetically. It is possible to use standard techniques to prepare a corresponding cDNA molecule from a corresponding mRNA.

While the said nucleic acid molecules can perfectly well contain additional base sequences (in order, for example, to encode a fusion protein), preferred embodiments relate to a nucleic acid molecule according to the invention which only possesses a base sequence which is selected from the group of the base sequences SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 4 and a base sequence which only differs from one of the said sequences because of the degeneracy of the genetic code, as explained above.

In another embodiment, nucleic acid molecules according to the invention additionally contain, at their C terminus, immediately after the end of the coding region, one or more stop codon(s). The naturally occurring stop codon, which was identified as being TAA, is preferred. However, the other known stop codons can also be used alternatively. It is also possible to use several stop codons.

The present invention also relates to a vector which contains one of the abovementioned nucleic acid molecules according to the invention. This vector is preferably suitable for transforming a host cell. This host cell is, in particular, a microorganism. This microorganism is preferably a filamentous fungus. Advantageously, the filamentous fungus is selected from the group consisting of Penicillium chrysogenum, Penicillium notatum, Penicillium brevicompactum, Penicilium citrinum, Acremonium chrysogenum, Aspergillus nidulans, Aspergillus niger, Aspergillus fumigatus, Aspergillus terreus and Tolypocladium inflatum. In a particularly preferred embodiment of the present invention, the microorganism or the filamentous fungus is Penicillium chrysogenum.

Such a vector can, for example, be present in the form of a plasmid. Such a vector contains, where necessary, other sequences in addition to a nucleic acid molecule according to the invention, for example an origin of replication and additional regulatory elements (promoter, translation start signal or termination signal, etc.) such that, after transformation has taken

place, the nucleic acid molecule according to the invention can be expressed. After transformation has taken place, a nucleic acid molecule according to the invention, as well as additional vector elements, can integrate into the genome of the host cell, with this corresponding to an amplification of the coding moiety of the novel gene. Advantageously, a vector according to the invention contains a nucleic acid molecule which comprises a base sequence as depicted in SEQ ID No. 4. Such a base sequence corresponds to the said SalI fragment and already contains regulatory sequences such as a corresponding promoter.

Standard techniques can be used to produce these vectors, by cloning a nucleic acid molecule according to the invention into suitable standard vectors.

The present invention furthermore relates to a host cell which has been transformed with a nucleic acid molecule according to the invention or with a vector according to the invention. This host cell is, in particular, a microorganism. This microorganism is preferably a filamentous fungus. Advantageously, the filamentous fungus is selected from the group which consists of Penicillium chrysogenum, Penicillium notatum, Penicillium brevicompactum, Penicilium citrinum, Acremonium chrysogenum, Aspergillus nidulans, Aspergillus niger, Aspergillus fumigatus, Aspergillus terreus and Tolypocladium inflatum. In a particularly preferred embodiment of the present invention, the host cell (or the microorganism or the filamentous fungus) is Penicillium chrysogenum.

Standard methods are used to transform such a host cell, in particular P. chrysogenum, with a vector according to the invention. An example of such a method is described in Austrian Patent Specification AT 391 481, Examples 6, 8, 10 and 12.

As an alternative to this, it is also possible to carry out what is termed a cotransformation. In this case, the vector containing a selection marker and the vector containing the gene according to the invention are used in the transformation as separate molecules.

As an alternative to this, the nucleic acid molecules according to the invention can also, in turn, be used for the transformation.

In particular, the genes to be introduced (the genes according to the invention and at least one marker gene) can consequently be used separately, for example as linear nucleic acid

molecules. A certain proportion of transformed host cells which harbour the vector to be selected, or the corresponding selection gene, then also contain the second gene which is employed in such a cotransformation. The proportion depends on the individual experiment and the experimental parameters which are selected in practice.

A transformed P. chrysogenum host cell according to the invention can advantageously be used for producing penicillin. The present invention consequently also relates to a process for producing penicillin, which process comprises culturing a P. chrysogenum host cell according to the invention under conditions which are suitable for bringing about the formation of penicillin by the host cell. Particular preference is given to selecting the penicillin from the group consisting of penicillin G and penicillin V.

Suitable culturing/fermentation techniques are known to the skilled person in the antibiotic field and have been used for a long time in producing penicillins.

In a preferred embodiment, the process according to the invention also comprises isolating the penicillin which has been formed. The penicillin which has been formed by a transformed P. chrysogenum host cell according to the invention can be purified and/or isolated from the fermented mycelium mash using known techniques, for example extraction with butyl acetate and subsequent chromatographic techniques.

Penicillin which has been produced in accordance with the invention, in particular penicillin G or penicillin V, can advantageously be converted into other derivatives having antibiotic properties.

An alternative application of the present invention relates to an isolated protein which comprises an amino acid sequence as depicted in SEQ ID No. 1. As mentioned, such a protein also encompasses corresponding fusion proteins from which, as desired, a mature protein having an amino acid sequence as depicted in SEQ ID No. 1 can be produced by means of cleavage. Preference is given to a protein according to the invention in which the protein only has the amino acid sequence as depicted in SEQ ID No. 1.

A protein according to the invention can be prepared by culturing a suitable prokaryotic or eukaryotic host cell, which harbours a suitable expression vector according to the invention

which contains a nucleic acid molecule encoding the protein, under conditions which bring about expression of the protein. The protein can be purified and isolated using customary techniques. Examples of suitable prokaryotic host cells in which a cDNA according to the invention is used, in particular, are bacterial cells, e.g. E. coli; examples of suitable eukaryotic host cells are yeast cells, such as Saccharomyces cerevisiae or Pichia pastoris, or mammalian cells, such as CHO or BHK cells.

A protein according to the invention can, for example, be used to convert, by means of a 4'-phosphopantethein group, appropriate enzymes, such as NRPS or PKS, or individual module or domain units thereof, from the apo form into the enzymically active holo form in vitro (see above). The protein according to the invention thus constitutes a valuable tool for preparing active in-vitro systems for generating new molecules, for example systems for combinatorial biosynthesis, from representatives of the said enzyme groups (such as NRPS or PKS) and also from other 4'-phosphopantethein-containing enzymes or individual moieties thereof.

The entire content of the scientific literature which is mentioned herein is hereby incorporated by reference.

The present invention is explained in more detail by means of the following examples; however, it is not restricted to these examples. The examples relate, in particular, to preferred embodiments of the present invention.

Examples

The materials and reagents which are mentioned herein are familiar to the skilled person, can be obtained commercially or are readily available and can be used in accordance with the manufacturers' instructions.

Example 1: Isolating the novel gene pptA from Penicillium chrysogenum

The gene according to the invention is prepared using the polymerase chain reaction. For this, DNA is isolated from the Penicillium chrysogenum strain ATCC48271. To do this, cells of the fungus are disrupted mechanically in liquid nitrogen by means of trituration in a mortar

and subsequently isolated using a standard technique, for example as described by T. Maniatis et al., Molecular Cloning - A Laboratory Manual, 1982, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA, or using a commercially available kit, for example as supplied by the company Qiagen.

An amplificate of approx. 3.2 kb in size is prepared, under standard conditions, from the genomic DNA using the primers PCR1f and PCR1r and a heat-stable DNA polymerase.

Primer PCR1f 5'- CCCC GTCGACCGAAGTGGTTTCGGTTCACTCGCACAT
(SEQ ID No. 5)

Primer PCR1r 5'- CCCC GTCGACGCGGGATTCGATGCTCAAAACTCTTGC
(SEQ ID No. 6)

For cloning the amplified region, which corresponds to the nucleic acid molecule according to the invention as depicted in SEQ ID No. 4, the fragment is cut with the restriction endonuclease SalI and ligated, by way of a SalI site, into an E. coli plasmid which is used in a standard manner; an example of such a standard plasmid is pBluescript II SK+ (from Stratagene).

The ligation product is transformed into E. coli (e.g. strain DH5alpha) and, in the E. coli, produced in a quantity which is sufficient for the subsequent step and then purified. Depending on the method of construction, it is also possible to obtain plasmids which contain the nucleic acid molecule in the reverse orientation; however, in principle, these structures function equally well.

Subsequent sequencing and analysis results in the nucleic acid sequences which are depicted in Figures 2 (SEQ ID No. 2) and 4 (SEQ ID No. 4). A cDNA sequence as shown in Figure 3 (SEQ ID No. 3) can then be deduced from them, as can the amino acid sequence of the encoded protein as shown in Figure 1 (SEQ ID No. 1). In principle, the cloning product can be conclusively verified by sequencing it and comparing the sequence with the DNA sequences shown in Figures 2 and/or 4. A plasmid which carries the SalI fragment is designated plasmid1 and then used.

Example 2: Functional characterization of the novel Penicillium chrysogenum gene pptA

In order to functionally characterize the novel gene, PCR is used to amplify the cDNA for the novel Penicillium chrysogenum pptA gene from P. chrysogenum total cDNA. The total cDNA is prepared from P. chrysogenum mRNA using commercially obtainable kits (e.g. from Qiagen) and standard laboratory methods.

The primers PCR2f and PCR2r are used to prepare an amplificate of approx. 1.25 kb in size from the cDNA, with this amplificate then being incorporated into the yeast vector pYES2.1-Sfi.

Primer PCR2f 5'- GGGGGCCGAGGCGGCCCATGGATACCAATGATATCAAACAG
(SEQ ID No. 7)

Primer PCR2r 5'- GGGGGCCATTATGGCCTCATTCAGGACTACCTGCCGCGAAACG
(SEQ ID No. 8)

The vector, and the subsequent implementation of the functional test, are described in detail in H.D. Mootz et al., "Functional chraracterization of 4'-phosphopantetheinyl transferase genes of bacterial and fungal origin by complementation of Saccharomyces cerevisiae lys5", FEMS Microbiol.Lett. 213 (2002), pp. 51-57. The yeast expression vector which is used for the functional test in the present example is prepared in analogy with pYES2-npgA (H.D. Mootz et al., see above, Chapter 2.2, page 53).

The test is based on the functional complementation of a particular defect in a yeast strain. The Lys5 gene encodes a PPTase which is essential for producing the amino acid lysine in the yeast cell and thus enables the yeast cell to grow on minimal medium without lysine. A specially constructed yeast strain in which the Lys5 gene has been destroyed can no longer produce lysine. The gene according to the invention, which has been incorporated into the abovementioned yeast expression vector, is transformed into this yeast strain. The test is described in detail in Chapter 3, pages 54-55, of the said publication by H.D. Mootz et al., see above.

Corresponding yeast transformants which contain the expressed P. chrysogenum pptA gene can grow once again on the selection medium (minimal medium without lysine), i.e. the lys5 defect is complemented (see also Chapter 3.4, page 55, in the said publication by H.D. Mootz et al.). This thereby demonstrates that the P. chrysogenum pptA gene is a gene encoding a functional 4'-phophopantetheinyl transferase.

Example 3: Cotransforming Penicillium chrysogenum

The nucleic acid molecule according to the invention described in Example 1 is prepared from an appropriate quantity (depending on the number of transformation assays to be carried out) of plasmid1, and made ready for transformation, by restricting the plasmid with SalI and then purifying the 3.2 kb fragment by means of agarose gel electrophoresis.

The P. chrysogenum niaD gene is used, as a selection marker, in the form of a constituent fragment of the plasmid J-12 which is described in Austrian Patent Specification AT 391 481. For this, plasmid J-12 is cut with EcoRI and the fragment of approx. 6.5 kb in size which carries the niaD fragment is ligated into the EcoRI-linearized plasmid pUCBM20 (Roche Diagnostics). This results in two possible plasmids which are in each case approx. 9.1 kb in size and which differ in the orientation of the incorporated EcoRI fragment. Corresponding plasmids from subclones are analysed by digesting them jointly with the enzymes XmaI and AgeI. A clone having the orientation where a fragment of approx. 2 kb in size and a fragment of approx. 7.1 kb in size are formed is selected and designated p1649A. The plasmid p1649A is cut with XmaI and AgeI and the fragment of approx. 7.1 kb in size is religated since XmaI and AgeI possess compatible ends. Plasmids from corresponding E. coli clones are examined by restricting them with EcoRI and designated p1649C.

A linear constituent fragment of plasmid p1649C, namely the EcoRI/XbaI fragment of approx. 4.5 kb in size, is used for transforming P. chrysogenum protoplasts of the corresponding strains. This linear constituent fragment is prepared by restricting the plasmid with the enzymes EcoRI and XbaI and then isolating the fragment. The fragment carries the niaD gene. It is naturally also possible to use the complete plasmids p1649C, p1649A or J-12 for the transformation in a corresponding manner.

In principle, it is possible to use any P. chrysogenum strains for which a suitable selection system is available as recipient strains for a transformation. A standard procedure for protoplast transformation is used to transform the two fragments which have been prepared, and which correspondingly contain the novel gene and, respectively, the niaD marker, into a P. chrysogenum strain (PC-180060) which is characterized as being an niaD mutant. Alternatively, a commercially available P. chrysogenum strain, such as ATCC48271 (designated P. chrysogenum strain P2) is used for the transformation.

The methodology of the protoplast transformation employed is described, for example, in Austrian Patent Specification AT 391 481 (see, in particular, Examples 6, 8, 10 and 12 in that publication) and involves generating a nitrate reductase mutant, transforming this mutant and subsequently selecting transformants on nitrate-containing nutrient agar. The properties of the niaD gene which is used for this selection are likewise described in the cited reference.

The protoplast density is adjusted to 10^8/ml in KCM buffer (0.7 M KCl/50 mM CaCl2/10 mM MOPS/pH 5.8). The aliquots of the solutions of the DNA fragments which are to be used for transformation are added to 100 µl of this suspension with the added volume being 10 µl. While the ratio of the two fragments is selected to be a molar ratio of from about 1-1.5 to 1, it is naturally also possible to add the fragments in another ratio. Approx. 1.5 – 3.5 µg of the approx. 4.5 kb EcoRI/XbaI fragment (containing the niaD gene) and approx. 0.8 – 1.8 µg of the approx. 3.2 kb SalI fragment from Example 1 (containing the gene according to the invention) are added per transformation assay sample. 50 µl of PEG (polyethylene glycol) solution (50 mM $CaCl_2$, 10 mM Tris, pH 7.5, 20% PEG) are then added and the whole is mixed and incubated on ice for 20 min. A further 0.5 ml of PEG solution (see above) are then added and the whole is carefully mixed by rotating the tube and then left to stand at room temperature for 5 minutes. After that, 1.5 ml of KCM buffer are added and the whole is once again carefully mixed. Finally, in each case about half of the transformation assay sample is mixed with 7 ml of R1 soft agar and poured onto the R1 selection agar (see also Austrian Patent Specification AT 391 481). After the agar plates had been incubated at 25°C for about two weeks, the transformant colonies had grown well and were available for further use.

Southern hybridization is used, for example, to test transformants from such experiments for the presence of additionally integrated copies of the gene according to the invention or of the essential plasmid1 moiety which was employed.

Example 4: Producing penicillin

Transformants generated in Example 3 are tested for penicillin production in flask fermentation experiments. Expediently, a population, which is of about the same size, of approx. 500-1000 cotransformants and transformants is in each case compared in parallel. To do this, supernatants from these flask fermentations are evaluated by HPLC analysis.

An appropriate method for penicillin G or penicillin V, depending on whether phenylacetate or phenoxyacetate has been added as precursor, is described, for example, in: C.S. Ho et al., "Enhancing Penicillin Fermentations by Increased Oxygen Solubility Through the Addition of n-Hexadecane", Biotechnology and Bioengineering 36 (1990), pp. 1110 – 1118.

The penicillin titers in the flask fermentations can be determined by means of HPLC analysis, for example as specified in L.H. Christensen et al., "A robust liquid chromatographic method for measurement of medium components during penicillin fermentations", Analytica Chimica Acta 296 (1994), pp. 51 – 62.

In order to obtain statistically relevant quantities of data, these analyses are repeated several times (e.g. 6 times) with in each case several (e.g. 4) parallel flask fermentations of each strain being carried out, and tested individually, at each repetition. Three strains (PC-20494, PC-20495 and PC-20496), which are derived from cotransformation with the pptA gene according to the invention and which exhibit a markedly higher penicillin productivity than does the starting strain, are identified in this way when starting with the P. chrysogenum strain PC-180060. These strains can be used on an industrial scale for the purpose of producing pencillins, in particular penicillin G or penicillin V. Appropriate fermentation processes for producing penicillins are known; see, for example, S. Queener and R. Swartz, in: A.H. Rose (Ed.), Secondary Products of Metabolism, Academic Press London, 1978, the chapter entitled "Penicillins: Biosynthetic and Semisynthetic"/the subchapter entitled "Fermentation", pages 50 – 74.

Standard published methods are used to extract the penicillin produced by the transformed strains from the fermentation mash and purify it (e.g. in accordance with S. Queener and R. Swartz, in: A.H. Rose (Ed.), Secondary Products of Metabolism, Academic Press London, 1978, chapter entitled "Penicillins: Biosynthetic and Semisynthetic"/subchapter entitled "Recovery of Penicillin", pages 75 – 86).